

DETAILED ACTION

1. Applicant's amendment and response filed 3/14/11 is acknowledged and has been entered.

2. Applicant is reminded of Applicant's election of Group I and species of ELISA plate as the substrate, antibody as the anchoring moiety, W6/32 as the antibody, as well as Applicant's election of the species of HLA-A2 with traverse in Applicant's amendment and response filed 12/1/06.

Claims 31-37, 45, 46, 48-51, 60 and 61 are currently being examined.

3. Applicant's amendment filed 3/14/11 has overcome the prior rejection of record of claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph, scope of enablement.

4. Applicant's amendment filed 3/14/11 has overcome the prior rejection of record of claim 42 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

5. Applicant is reminded that for the purpose of prior art rejections, the filing date of the instant claims is deemed to be the filing date of the instant application, *i.e.*, 9/24/03, as the parent applications do not support the claimed limitations of the instant application. The provisional parent application serial no. 60/413,842 only discloses ELISA assays using W6/32 or pan-HLA antibody immobilized HLA to detect anti-HLA antibodies. The provisional parent application serial no. 60/474,655 discloses some aspects of making soluble HLA from gDNA or cDNA. The parent application serial no. 10/337,161 and 10/022,066 disclose soluble HLA and making soluble HLA, respectively. In addition, the provisional parent applications do not disclose "pool" in the context of the claimed method.

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

7. Claims 31-37, 45, 46, 49-51, 60 and 61 are rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 5,482, 841 (IDS reference) in view of McClusky *et al* (J. Immunol. 1988 141: 1451-1455, of record), Prilliman *et al* (Immunogenetics, 1997,

Art Unit: 1644

45: 379-385, IDS reference), DiBrino *et al* (Biochemistry 1995, 34(32):10130-10138, of record), Hausmann *et al* (Clin. Exp. Immunol. 1993, 91: 183-188, of record) and Chen *et al* (J. Immunol. 1994, 152: 2874-2881, of record).

The instant claims were previously rejected over this combination of references in the prior Office Action of record. However, the instant rejection has been modified to take into account the newly added claim limitation recited in instant base claim 31, *i.e.*, "harvesting the soluble class I MHC complexes from the culture while retaining the mammalian cell line in culture for the production of additional soluble class I MHC complexes" and to delete claim 42 which has been canceled.

U.S. Patent No. 5,482, 841 discloses an assay method for detecting the presence of anti-HLA antibodies in a sample, said assay comprising use of HLA molecules extracted from cells and purified by detergent extraction (*i.e.*, full-length MHC class I molecules that comprise the TM and cytoplasmic regions), centrifugation, PEG and NH_4SO_4 precipitation, said HLA molecules indirectly linked to a solid support such as beads, membranes and microtiter plates by polyclonal or monoclonal antibodies specific for the $\alpha 3$ domain of Class I HLA or the associated $\beta 2m$ chain or to a conformational epitope expressed by the combination of both chains, or specific to epitopes conserved across a class or subset of HLA molecules, such as ones specific for HLA-A, B or C. Thus, the art reference inherently discloses that the full-length, detergent-extracted HLA complexes are occupied with endogenous peptides (*i.e.*, because of the isolation protocol, the HLA molecules retain their conformation that is dependent upon peptide being bound in the HLA binding groove, as evidenced by detection with conformation dependent anti-HLA antibodies).

U.S. Patent No. 5,482, 841 further discloses that a sample containing antibodies is added, then bound antibodies are separated from free antibodies and other non-specifically bound proteins or other components, and the presence of the antibodies is detected using a labeled reagent such as anti-human antibody against IgG, IgM or IgA. U.S. Patent No. 5,482, 841 discloses that the samples may be biological fluids such as blood, CSF, tears, saliva, lymph, dialysis fluid, organ or tissue culture derived fluids and fluids extracted from physiological tissues. U.S. Patent No. 5,482, 841 discloses that of particular interest are allo-antibodies found in the serum of transplant or prospective transplant patients, and that the determination of the presence and specificity of antibodies against foreign HLA antigens is therefore clinically important for monitoring transplant patients, and the assay may test for reactivity against a panel of antigens or may be specific for a single donor. U.S. Patent No. 5,482, 841 discloses that the solid support can be microtiter plates (with wells), glass, plastic, polysaccharides, nylon or nitrocellulose [membranes] or paramagnetic component materials surrounded by plastic. U.S. Patent No. 5,482, 841 discloses using negative and positive control samples. U.S. Patent No. 5,482, 841 discloses a kit for use in a method for detecting at least one receptor analyte specific for an HLA antigen in a biological sample, said kit comprising a solid support coated with a capture agent capable of specifically binding to

Art Unit: 1644

a conserved region of a subset of interest of HLA antigens and a labeled reagent that specifically binds to human antibodies, and wherein the capture agent may be an antibody directed to the $\alpha 3$ domain of HLA class I heavy chain (see entire reference).

U.S. Patent No. 5,482, 841 does not disclose wherein the HLA molecules in the assay method are those produced by the method steps recited in the instant claims, *i.e.*, wherein the HLA class I molecules are soluble, truncated molecules that consist of the class I MHC heavy chain and endogenous $\beta 2m$ and peptide from the mammalian host cell.

U.S. Patent No. 5,482, 841 does not disclose wherein the MHC class I heavy chains are obtained by isolating mRNA from a source, wherein the mRNA encodes an MHC heavy chain allele, reverse transcribing the mRNA to obtain cDNA and identifying an individual MHC class I heavy chain allele in the cDNA.

McClusky *et al* teach that their complex of soluble class I heavy chain (a soluble murine MHC class I heavy chain consisting of the $\alpha 1\alpha 2$ regions of H-2D^d and the $\alpha 3$ and carboxyl-terminus of Q10^b (which is a secreted protein)) plus the non-covalently-associated $\beta 2m$ from the host cell (L cells) is especially valuable because it is a monovalent, soluble, homogenous, recombinant protein, free of detergent, lipid or cellular membrane components. McClusky *et al* teach isolation of the complex using anti-H-2D^d antibodies coupled to Sepharose-4B, subsequent elution from the immunoaffinity column at pH 11.5 and subsequent neutralization. McClusky *et al* teach that the antigen-specific TCR of effector T lymphocytes recognizes antigenic peptide in the context of self MHC class I and that consequently, such complexes are useful for the study of MHC/TCR interactions (especially second full paragraph at column 1 on page 1454, reference 8, materials and methods and introduction sections, and last paragraph of reference).

Thus, McClusky et al teach producing an intact, soluble chimeric murine MHC class I complex occupied with endogenous peptide in the binding groove that is especially valuable because it is a monovalent, soluble, homogenous, recombinant protein, free of detergent, lipid or cellular membrane components, but do not teach truncating a non-chimeric class I MHC heavy chain to exclude the transmembrane and cytoplasmic domains using a PCR primer.

Prilliman *et al* do provide this teaching as follows, as well as method steps recited in the instant claims, exclusive of those involving mRNA. Prilliman *et al* teach large-scale production of Class I HLA in roller bottles (*i.e.*, in a large scale mammalian tissue culture system) for expansion of transfected cells for inoculation into a hollow fiber bioreactor unit for high yield production of soluble class I HLA. Prilliman *et al* teach a full-length, single stranded cDNA clone of HLA-B*1501 used as a template in PCR amplification with primers, the 3' primer introducing a TGA stop codon, truncating the expressed form of the molecule through removal of the transmembrane and cytoplasmic exons from the

Art Unit: 1644

coding region. Prilliman *et al* teach the pCR product is directionally subcloned into M13, and then subcloned into the mammalian pBJ1-neo expression vector, and the resulting construct is transfected into the class I-negative EBV-transformed lymphoblastoid line 721.221. The soluble class I HLA occupied with endogenous peptide is then collected from the bioreactor and adsorbed onto a BBM.1 (anti- β 2m) antibody-Sepharose 4B column. Further downstream processing of the complexes (*i.e.*, extraction of the peptides from the HLA complexes) deviates from that taught by McClusky *et al*, as Prilliman *et al* did not wish to maintain the integrity of the complexes.

Thus, Prilliman et al teach that soluble human class I MHC complexes consisting of MHC class I heavy chain, β 2m light chain and occupied with endogenous peptides can be produced by creating constructs that lack the coding regions for the transmembrane and cytoplasmic domain, can be produced as soluble molecules in high yield in a hollow fiber bioreactor unit, and can be adsorbed onto a mAb-affinity column and eluted from said column. Prilliman, do not, however, teach isolating mRNA, nor do they teach isolating the affinity-purified class I MHC complexes without further downstream processing.

DiBrino *et al* do provide the teaching of isolating mRNA encoding a truncated class I MHC heavy chain. DiBrino *et al* teach making a truncated HLA class I heavy chain lacking the transmembrane and cytoplasmic regions by PCR amplification of an HLA-B44 cDNA clone using specific primers and a prokaryotic *E. coli* plasmid expression vector (amino acid residues 1-278), that can be combined *in vitro* with β 2m light chain and peptide to form MHC class I complexes. DiBrino *et al* also teach obtaining and full length cDNA for HLA-B*4403 by PCR amplification of cDNA made from RNA isolated from the immortalized human lymphoblastoid B cell line W1B. The cDNA was sequenced to confirm identity of the heavy chain, cloned into the mammalian expression vector RSV.neo and transfected into Hmy2.C1R cells, where they are expressed on the cell surface in complex with β 2m and endogenous peptides (class I deficient cell line). DiBrino *et al* teach detection of said HLA using W6/32 monoclonal antibody specific for human Class I molecules (especially materials and methods section).

Thus, DiBrino et al teach that the heavy chain of a MHC class I molecule can be constructed so that it lacks the TM and cytoplasmic regions, and that the heavy chain is capable of associating in vitro with β 2m light chain and peptide to form MHC class I complexes. DiBrino et al also teach that the full-length heavy chain encoded construct, when transfected into a mammalian host cell, can associate with β 2m light chain, acquire endogenous peptide and be transported to the cell surface.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have produced a soluble MHC class I complex similar to the complex disclosed by U.S. Patent No. 5,482,841, except instead of a full-length molecule, making one that comprises a truncated version of an MHC class I, said

Art Unit: 1644

truncated version being a soluble intact class I MHC molecule complex consisting of the MHC class I heavy chain extracellular regions and lacking the TM and cytoplasmic domains, and $\beta 2m$ and endogenous peptide from the host cell, using the 3' primer type taught by Prilliman *et al* or DiBrino *et al*, and the inserting, electroporating, inoculating, culturing and immunoaffinity purification steps taught by Prilliman *et al* (*i.e.*, creating a mammalian plasmid encoding vector for a truncated heavy chain lacking the transmembrane and cytoplasmic regions, transfecting the expression vector into a suitable host cell, inoculating a hollow fiber bioreactor, harvesting the soluble class I MHC complexes from the said hollow fiber bioreactor unit, and isolating them by immunoaffinity purification as intact complexes), but starting with mRNA as taught by DiBrino *et al*.

One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to produce large quantities of soluble, truncated and intact MHC class I complexes as taught by Prilliman *et al* to be used in the method for anti-MHC class I antibody detection as disclosed by U.S. Patent No. 5,482,841, particularly in light of the teaching of McClusky *et al* that a secreted version of MHC class I complexes is particularly valuable because it is a monovalent, soluble, homogenous, recombinant protein that is free of detergent, lipid or cellular membrane components.

Hausmann *et al* teach isolated HLA-A2 complexes that have been papain solubilized (*i.e.*, they are soluble and lack the transmembrane and cytoplasmic domains) from the MHC-class I bearing EBV-transformed spleen cell line M4 (*i.e.*, a mammalian cell line) and isolated by affinity chromatography with anti-HLA-A2 antibodies (with elution at pH 11.5 prior to neutralization), wherein the complexes comprise the MHC class I heavy chain extracellular regions without the transmembrane and cytoplasmic domains, non-covalently associated $\beta 2m$ and an endogenously loaded MHC class I binding peptide bound in the peptide binding groove. Hausmann *et al* teach that these class I MHC complexes are useful for the study of T cell reactivity against foreign MHC products in transplantation settings, as well as for study of immunomodulation (especially abstract, introduction, materials and methods, page 184, column 1, first two paragraphs, paragraph spanning pages 185-186).

Chen *et al* teach that elution at pH 11.5 of MHC class I molecules from anti-class I antibody affinity columns does not dissociate the complex, nor elute endogenous peptides from the antigen binding groove of MHC class I molecules (especially page 2875 at the paragraph spanning columns 1-2 and the paragraph spanning pages 2876-2877).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have collected the immunoaffinity purified intact complexes taught by Prilliman *et al* without subjecting them to the further downstream processing that is taught by Prilliman *et al*.

Art Unit: 1644

One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to produce intact complexes as taught by Hausmann *et al*, especially since Hausmann *et al* teach that these intact complexes are useful for study of alloreactivity.

In addition, the instant claims are included in this rejection because although Prilliman *et al* do not explicitly teach wherein the soluble class I HLA complexes after harvesting and acid elution from the anti- β 2m-Sepharose 4B immunoaffinity complexes are intact complexes with the conformation of the native complexes prior to said step, said art reference does teach that further acidification is required to denature the class I heavy chain- β 2m-peptide complexes in order to liberate the bound peptides. Therefore, the claimed process appears to be similar to the process of the prior art absent a showing of unobvious differences. Since the Patent Office does not have the facilities for examining and comparing the process of the instant invention to those of the prior art, the burden is on Applicant to show an unobvious distinction between the process of the instant invention and that of the prior art. See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977).

Alternatively, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have eluted the HLA class I complexes from the immunoaffinity column by any art known method that would preserve the conformation and integrity of the complexes, such as by the methodology taught by Hausmann *et al* and Chen *et al*.

One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to generate soluble forms of HLA class I molecules to for the assay disclosed by the primary reference.

It would have also been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used the human class I MHC-specific W6/32 mAb taught by DiBrino *et al* in the immunoaffinity purification.

One of ordinary skill in the art at the time the invention was made would have been motivated to do this because DiBrino *et al* teach that this mAb is specific for human class I MHC, Prilliman *et al* teach using a class I MHC negative mammalian host cell in order to produce the soluble, truncated MHC class I molecules, and DiBrino *et al* teach using a class I MHC deficient cell line for this same purpose, *i.e.*, W6/32 can bind to all human class I MHC molecules, but only one specific soluble MHC class I allele product is being produced and isolated.

Claim 46 is included in this rejection because it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have optimized the method of expression by insuring that the mammalian expression vector contains a

Art Unit: 1644

promoter that facilitates increased expression of the PCR product, as was routine in the art.

With regard to the newly recited limitation in instant base claim 31 “retaining the mammalian cell line in culture for production of additional soluble class I MHC complexes”, Prilliman *et al* teach producing the soluble class I MHC molecules in a bioreactor that retains the mammalian cell line for production of additional soluble class I MHC molecules, as the molecules are secreted while the cells are retained.

Applicant's arguments have been fully considered but are not persuasive.

Applicant's arguments are of record in the response filed 3/14/11 on pages 8-16. However, Applicant is arguing the references separately. In response to Applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). With further regard to this, and to Applicant's argument about the disadvantages of using detergent solubilization and detergent solubilization constituting a teaching away, McClusky *et al* cited in the instant rejection teach that soluble MHC is valuable because it is a monovalent, soluble, homogenous, recombinant protein, free of detergent, lipid or cellular membrane components, providing motivation to avoid detergent solubilization.

Applicant argues that there is no *teaching in* McClusky *et al* for how a secreted protein is to be created. Prilliman *et al* and DiBrino *et al*, cited in the instant rejection, provide such a teaching as enunciated in the instant rejection, and these constructs do not contain a TM or cytoplasmic region. Applicant is arguing the McClusky *et al* reference separately in arguing that McClusky *et al* teach the formation of a hybrid molecule and that said group does not explore the nature of the truncation site to see if secretion can be obtained without using the Q10 molecule. Prilliman *et al* provide said teaching, their class I MHC molecule lacking TM and cytoplasmic regions being secreted. With respect to this and Applicant's further arguments about the senior author of McClusky *et al* publication, Applicant's said arguments are therefore off-point. Likewise, Applicant's argument that McClusky *et al* teach away from the claimed method in which the TM and cytoplasmic regions have been removed, is also found non-persuasive for the reasons enunciated below.

Applicant also argues the references separately in arguing that the art teaches away from the step of harvesting soluble class I MHC complexes while retaining some host cells in culture for production of additional soluble class I MHC complexes. Prilliman *et al* teach this limitation. (See below for further discussion).

Prilliman *et al* teach large-scale production of class I HLA molecules truncated to exclude the TM and cytoplasmic domains in a hollow fiber bioreactor. Although Prilliman *et al* teach further downstream processing of the complexes (*i.e.*, extraction

Art Unit: 1644

of the complexes from the immunoaffinity column in a way that destroys the integrity of the complexes in order to obtain the MHC-bound peptides), the primary art reference '841 discloses the usefulness of intact soluble class I MHC/HLA molecules and exemplifies extracting detergent solubilized HLA molecules, McClusky *et al* also teach the usefulness of intact soluble class I MHC molecules produced from gDNA, but also teach that recombinantly produced intact soluble class I MHC molecules are especially valuable because they are monovalent, soluble, homogenous protein, free of detergent, lipid or cellular membrane components. DiBrino *et al*, like Prilliman *et al*, teach that the heavy chain of a MHC class I molecule can be constructed so that it lacks the TM and cytoplasmic regions, and furthermore, that the heavy chain is capable of associating with β 2m light chain and peptide to form MHC class I complexes. Hausmann *et al* and Chen *et al* teach a method of elution of MHC class I molecules from immunoaffinity columns that leaves the complexes intact. The combination of references provides the motivation and the method to make intact, soluble MHC/HLA class I molecules from gDNA without further downstream processing taught by Prilliman *et al* that destroys the complexes, for the use disclosed by '841.

In response to Applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the Applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

In response to Applicant's argument that the examiner has combined an excessive number of references, reliance on a large number of references in a rejection does not, without more, weigh against the obviousness of the claimed invention. See *In re Gorman*, 933 F.2d 982, 18 USPQ2d 1885 (Fed. Cir. 1991).

8. Claim 48 is rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 5,482, 841 (IDS reference) in view of McClusky *et al* (J. Immunol. 1988 141: 1451-1455, of record), Prilliman *et al* (Immunogenetics, 1997, 45: 379-385, IDS reference), DiBrino *et al* (Biochemistry 1995, 34(32):10130-10138, of record), Hausmann *et al* (Clin. Exp. Immunol. 1993, 91: 183-188, of record) and Chen *et al* (J. Immunol. 1994, 152: 2874-2881, of record) as applied to claims 31-37, 45, 46, 49-51, 60 and 61 above, and further in view of Nakajima and Yaoita (Nucleic Acids Res. 1997, 25(11): 2231-2232, of record).

U.S. Patent No. 5,482,841, Hausmann *et al*, Prilliman *et al* and DiBrino *et al* have been discussed supra.

Art Unit: 1644

The combination of U.S. Patent No. 5,482,841, Hausmann *et al*, Prilliman *et al* and DiBrino *et al* does not teach wherein the locus-specific primer includes a sequence encoding a tail that is used to facilitate purification of the soluble class I MHC complexes.

Nakajima and Yaoita teach that the advantage of epitope tagging a recombinant protein using PCR is that by adjoining an epitope peptide to a protein of interest, a commercially available antibody to the epitope substitutes for an antibody to a recombinant protein (see entire reference, especially abstract and first paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have added a epitope tag (or 'tail') to the encoded MHC class I heavy chain after the $\alpha 3$ extracellular region.

One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to facilitate purification of MHC complexes using a purification tail at the 3' end, without interfering with peptide binding at the 5' end of the heavy chain that forms the peptide binding groove.

Applicant's arguments have been fully considered but are not persuasive.

Applicant's arguments are of record in the response filed 3/14/11 on pages 8-16.

Applicant argues that Nakajima and Yaoita do nothing to supply the alleged deficiencies of the rejection of the independent claim.

However, the Examiner's response to Applicant's arguments enunciated supra, also apply herein.

9. No claim is allowed.

10. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Art Unit: 1644

11. Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Marianne DiBrino whose telephone number is 571-272-0842. The Examiner can normally be reached on Monday, Tuesday, Thursday and Friday.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Phuong "Neon" Huynh, can be reached on 571-272-0846. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Marianne DiBrino, Ph.D.
Patent Examiner
Group 1640
Technology Center 1600

/G. R. Ewoldt/
Primary Examiner, Art Unit 1644